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**Lack of genetic structure and evidence for long-distance dispersal
in ash (*Fraxinus excelsior*) populations under threat from an
emergent fungal pathogen: implications for restorative planting**

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Abstract Genetic analysis on populations of European ash (*Fraxinus excelsior*) throughout Ireland was carried out to determine the levels and patterns of genetic diversity in naturally seeded trees in ash woodlands and hedgerows, with the aim of informing conservation and replanting strategies in the face of potential loss of trees as a result of ash dieback. Samples from 33 sites across Northern Ireland and three sites in the Republic of Ireland were genotyped for eight nuclear and ten chloroplast microsatellites. Levels of diversity were high (mean $A_R = 10.53$; mean $H_O = 0.709$; mean $H_E = 0.765$), and were similar to those in Great Britain and continental Europe, whilst levels of population genetic differentiation based on nuclear microsatellites were extremely low ($\Phi_{ST} = 0.0131$). Levels of inbreeding (mean $F_{IS} = 0.067$) were significantly lower than those reported for populations from Great Britain. Fine-scale analysis of seed dispersal indicated potential for dispersal over hundreds of metres. Our results suggest that ash woodlands across Ireland could be treated as a single management unit, and thus native material from anywhere in Ireland could be used as a source for replanting. In addition, high potential for dispersal has implications for recolonization processes post-ash dieback (*Chalara fraxinea*) infection, and could aid in our assessment of the capacity of ash to shift its range in response to global climate change.

ADDITIONAL KEYWORDS: Gene flow, genetic diversity, inbreeding, microsatellites, spatial genetic structure, replanting

Introduction

In recent years, many ecologically and economically important tree species have come under threat from a range of emergent pathogens. The outbreaks of the fungus *Ophiostoma novo-ulmi*, the agent of Dutch elm disease in the 1900s, led to extensive losses of several *Ulmus* species, including an estimated two-thirds of the elm population of the UK during the 1970s (Webber 1981). In the last decade in the UK and Ireland, notable fungal and oomycete pathogens have included sudden oak death, chestnut blight and red needle blight. Most recently, outbreaks of ash dieback, a potentially serious threat which affects several species of ash (*Fraxinus* spp.), have been reported in continental Europe, and have subsequently spread to Great Britain and Ireland. Common or European ash (*F. excelsior*) is a key species of mixed broadleaved woodlands across Europe, with a natural range that extends from southern Scandinavia to northern Spain and the Balkans, and from Ireland in the west to continental Russia in the east. European ash within woodlands forms mixed stands, usually with beech (*Fagus sylvatica*), pedunculate oak (*Quercus robur*), sessile oak (*Q. petraea*), alder (*Alnus glutinosa*) and sycamore (*Acer pseudoplatanus*), and is an important component of woodland ecosystems, as well as being a valuable timber species (FRAXIGEN 2005). The symptoms of ash dieback were first reported in Poland in the early 1990s (Pautasso et al. 2013), but it was not until 2006 that the causative agent of ash dieback was identified as *Chalara fraxinea* (Kowalski 2006), which has since been found to be synonymous with the ascomycete fungus *Hymenoscyphus pseudoalbidus* (Queloz et al. 2011). The disease was first recorded in Britain in February 2012, and the first case of ash dieback in Ireland was reported in October 2012.

Replanting of forests will have to be considered if ash dieback outbreaks result in substantial loss of trees, either via pathogenic mortality or anthropogenic clearance to prevent

possible spread. In Great Britain, the Forestry Commission has developed recommendations to maintain provenance of replanted individuals, by using seed sourced from the same area (Herbert et al. 1999). Consequently, a map of “seed zones” that divide Great Britain into 24 areas delineated by geographic features and general climatic similarity has been drawn up to assist restorative conservation programmes. However, a recent study on ash in England, Scotland and Wales (Sutherland et al. 2010) found limited genetic differentiation between 42 populations from 21 of the 24 seed zones, indicating large-scale genetic homogeneity. This suggests that all populations of ash in Britain could be treated as a single management unit (DeSalle and Amato 2004), a more efficient and cost-effective approach to replanting, contrary to recommendations based on previously identified “seed zones”.

Seed dispersal plays a central role in the demography of natural plant populations across a broad range of geographic scales, from initial colonization to shaping community structure and regeneration (Howe and Smallwood 1982; Nathan and Muller-Landau 2000; Levine and Murrell 2003). Despite the importance of dispersal in plant population ecology, the logistics of tracing dispersal events accurately from source are not straightforward. Methods involving “tagging” of seeds are generally less than optimal due to factors such as extremely low recovery rates and the effects of the tags themselves on the dispersal process (reviewed in Wang and Smith 2002; Forget and Wenny 2005; Ashley 2010). Most attempts to estimate seed dispersal distributions have instead relied on seed trapping, coupled with models that generally make *a priori* assumptions about seed source (Nathan and Muller-Landau 2000). In recent years, climate change, habitat loss and fragmentation, and increased mortality associated with emergent plant pathogens, such as *H. pseudoalbidus*, have increased interest in more direct, precise measurements of seed dispersal to determine the capacity of plant populations to recover from these threats.

69 With the recent report of the first case of ash dieback in Ireland, and the lack of population
70 genetic information for the species across the island, the main aim of the present study was to
71 determine the levels and patterns of genetic diversity in naturally seeded trees in ash
72 woodlands and hedgerows. We focused on Northern Ireland which, like the rest of Ireland,
73 has no map of “seed zones” on which to base management units, and the development of
74 rational conservation and replanting strategies. We analysed populations from the northern,
75 eastern, southern and western extremes of Ireland to ensure our findings are applicable to the
76 island as a whole. We also used a molecular genetic approach to quantify fine-scale seed
77 dispersal distances in two natural ash woodlands, employing a combination of high-resolution
78 nuclear and chloroplast microsatellite markers. Our results suggest that ash woodlands across
79 Ireland could be treated as a single management unit, and thus material from anywhere in
80 Ireland could be used as a source for replanting. We also identified potential for seed
81 dispersal over hundreds of metres, which will be important in addressing both post-ash
82 dieback recolonization, and assessing the capacity of ash to migrate in response to global
83 climate change.

Materials and methods

Study species reproductive ecology

European ash (*Fraxinus excelsior*) has protandrous, anemophilous flowers. The species exhibits a wide range of complex polygamy, ranging from pure male and female trees, through combinations of male / female and hermaphroditic flowers in the same individual, to sexual changes across successive years (Wardle 1961; Bacles and Ennos 2008). Although *F. excelsior* is preferentially outcrossing, hermaphrodites are self-compatible, and whereas females and hermaphrodites exhibit high seed set, hermaphrodites exhibit reduced male fertility. Fruits are winged and wind-dispersed, and generally contain a single seed. Seedlings are shade-tolerant, but need good light levels to promote full growth, generally only establishing in clearings within woodlands (Marigo et al. 2000).

Sampling and DNA extraction

For the broad-scale study, samples were collected from 33 sites across Northern Ireland and three sites in the Republic of Ireland that had been previously designated as ancient or semi-natural woodland based on data collected for the Woodland Trust Inventory of ancient and long-established woodland in Northern Ireland (www.backonthemap.org.uk) and the National Survey of Native Woodlands 2003-08 in the Republic of Ireland (www.npws.ie; Fig. 1 and Table 1). The congeneric *F. angustifolia* has been planted in the Republic of Ireland, but is not found in the vicinity of any of the native woodlands analyzed in the present study. A single leaf was collected from each of 30 trees per site and stored in silica gel, and GPS coordinates recorded for every tree sampled. DNA was extracted using the CTAB method of Doyle and Doyle (1987).

For the fine-scale study, two sites were chosen. The first, Barnett Demesne, was also used for the broad-scale study. It is a *ca.* 40 ha public park in South Belfast, Northern Ireland (54.55° N, 5.96° W – Fig. 2), and is an area of mixed parkland and woodland, the woodland being semi-continuous stands of mixed deciduous trees, primarily beech and oak. The ash is found in the northern part of the main wooded area, with a few scattered trees in the adjoining parkland. The second site, Cregagh Glen, is a narrow (50 – 60 m), steep-sided ravine *ca.* 700 m long on the eastern outskirts of Belfast (54.56° N, 5.89° W – Fig. 2). It is the surviving remnant of a former *ca.* 400 ha forest and comprises mixed woodland of Scots pine, sycamore, beech and ash. The ash is distributed sporadically throughout the length of the Glen. For both sites, samples were obtained from all reproductive (adult) trees, as well as from selected saplings (96 from Barnett Demesne and 48 from Cregagh Glen; Figure 2). A single leaf was collected from each individual and stored in silica gel, and GPS coordinates recorded (Table S1, Supporting Information). DNA was extracted using the CTAB method of Doyle and Doyle (1987).

Genotyping

All trees and saplings were genotyped for eight nuclear and ten chloroplast microsatellite loci. For nuclear microsatellite genotyping, we used six previously reported loci which have been widely used in population genetic studies on ash: Femsatl-4, Femsatl-8, Femsatl-11, Femsatl-16 and Femsatl-19 (Lefort et al. 1999) and M230 (Brachet et al. 1999), as well as two loci developed for the present study (FR639485 and FR646655). As previous studies highlighted the possibility of null alleles using the Lefort et al. (1999) and Brachet *et al.* (1999) primers (Morand et al. 2002; Ferrazzini et al. 2007; Sutherland et al. 2010), we designed new primers for all loci (Table 2) using the Primer3 program (v 0.4.0; <http://primer3.ut.ee>). The F_{IS} values calculated in the present broad-scale study were lower

than those from several previous studies, which is consistent with the occurrence of null alleles when using the original primers. To investigate this further, we also genotyped a subset of our samples for comparison using the original Femsatl-4, Femsatl-8 and Femsatl-16 primers, since these exhibited the highest F_{IS} values in the earlier studies. To develop further markers, we also tested five pairs of primers developed from EST sequences in GenBank, but only two of these (FR639485 and FR646655) consistently gave clear, reproducible products.

Fraxinus excelsior chloroplast sequences in the GenBank database were searched for mononucleotide repeats of ten or more (Provan et al. 2001). Primers were designed using the Primer3 program to amplify the ten loci in four multiplexes (Table S2, Supporting Information). One of these (AF528042.2) corresponds to the highly polymorphic CPFRA6 locus described in Harbourne et al. (2005), but was monomorphic across all samples tested. Consequently, we screened a subset of our samples using the original CPFRA6 primers, but these did not reveal any additional variation to that displayed using the AF528042.2 primers.

PCR was carried out in a total volume of 10 μ l containing 100 ng genomic DNA, 5 pmol of 6-FAM- or HEX-labelled M13 primer, 0.5 pmol of M13-tailed forward primer, 5 pmol reverse primer, 1x PCR reaction buffer, 200 μ M each dNTP, 2.5 mM $MgCl_2$ and 0.25 U GoTaq Flexi DNA polymerase (Promega, Sunnyvale, CA, USA). PCR was carried out on a MWG Primus thermal cycler (Ebersberg, Germany) using the following conditions: initial denaturation at 94 °C for 3 min followed by 40 cycles (30 for chloroplast loci) of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s, extension at 72 °C for 30 s, and a final extension at 72 °C for 5 min. Genotyping was carried out on an AB3730xl capillary genotyping system. (Applied Biosystems, Foster City, CA, USA). Allele sizes were scored using the GENEMAPPER software package (v4.1; Applied Biosystems) using LIZ-500 size standards, and were checked by comparison with previously sized control samples. Chromatograms were all inspected visually to check for large allele dropout (see Discussion).

Data analysis –broad-scale

GENEPOP (V3.4; Raymond and Rousset, 1995) was used to test for linkage disequilibrium between nuclear microsatellite loci. To estimate genetic diversity within the populations, levels of observed (H_O) and expected (H_E) heterozygosity, levels of allelic richness (A_R) and fixation indices (F_{IS}) were calculated using the FSTAT software package (V2.9.3.2; Goudet, 2001). Significance of F_{IS} was determined by 10,000 randomisation steps. We also estimated null allele frequencies using the CERVUS software package (V3.0.3; Kalinowski et al. 2007), as previous studies using the same microsatellites (Femsatl-4, Femsatl-8, Femsatl-11, Femsatl-16, Femsatl-19 and M230) have suggested the possibility of null alleles. Chloroplast microsatellite allele sizes were combined into haplotypes, and levels of genetic diversity (H) based on haplotype frequencies were calculated using the ARLEQUIN software package (V3.5.1.2; Excoffier and Lischer, 2010).

The overall level of genetic differentiation between populations was estimated using Φ_{ST} , which gives an analogue of F_{ST} (Weir and Cockerham, 1984) calculated within the analysis of molecular variance (AMOVA) framework (Excoffier et al. 1992) using ARLEQUIN. In addition, as the high numbers of alleles and high levels of diversity associated with microsatellite loci can lead to an underestimation of genetic differentiation between populations, we also calculated Hedrick's G'_{ST} (Hedrick 2005) for the nuclear microsatellite data set. To further identify possible patterns of genetic structure, the software package BAPS (V5; Corander et al. [2003]) was used to identify clusters of genetically similar populations using a Bayesian approach. Ten replicates were run for all possible values of the maximum number of clusters (K) up to $K = 36$, the number of populations sampled, with a burn-in period of 10,000 iterations followed by 100,000 iterations. Multiple independent runs always gave the same outcome.

A test for isolation-by-distance (IBD; Rousset 1997) was carried out to test the null hypothesis of a stepping-stone model of gene flow between populations of *F. excelsior*. The ISOLDE test implemented in the GENEPOP software package was used to assess the relationship between genetic distance, measured as Hedrick's G'_{ST} (Hedrick 2005), and geographical distance between population pairs. 1,000 permutations were used for the Mantel test.

To test for spatial genetic structuring (SGS) within populations, which could give rise to Wahlund effects, we carried out spatial autocorrelation analyses using SPAGeDI (V1.4; Hardy and Vekemans, 2002). Mean coancestry coefficients (θ_{xy} ; Loiselle et al. 1995) between pairs of individuals were calculated for both the 0-50 m and 50-100 m distance classes for each population, with the remaining size intervals (50 m to 500 m) reflecting the overall size of each population, and plotted as a correlogram, with 95% confidence intervals calculated from 1,000 permutations of individuals within each distance class, and for estimates of θ_{xy} using 1,000 permutations. Finally, for comparison of levels of SGS with other species, we calculated the S_p statistic of Vekemans and Hardy (2004) as $-b_k / (1 - \theta_1)$, where b_k is the slope of the regression of θ_{xy} against the logarithm of the distance, and θ_1 is the mean value of the pairwise coancestry coefficients calculated between all pairs of individuals within the first distance class (0-50 m).

Data analysis – fine-scale

Only seven nuclear microsatellite loci were used in the fine-scale study, since locus Femsat-19 exhibited alleles that differed by only a single base pair, and we wanted to ensure exact matches between putative parents and offspring. We employed two approaches to determine parentage of saplings. The first was a simple exclusion approach, based on the premise that we had sampled all potential adult parents in each stand. Any adult that did not have at least

207 one allele matching those exhibited by a sapling at all seven loci was excluded as a potential
208 parent of that sapling. The second was a likelihood-based approach implemented in the
209 CERVUS software package (V3.0; Kalinowski et al. 2007). This was used in addition to strict
210 exclusion, since the program can allow for potential genotyping errors, and the fact that not
211 all putative parents may have been sampled. Simulations were run for 10,000 iterations, with
212 a genotyping error rate of 0.01, since we had manually scored all markers to check for
213 automated miscalls and allelic dropout, and assuming 95% sampling of putative parents.
214 Parent-pairs or individual parents were assigned based on the critical values for the 95% strict
215 log-likelihood (LOD) scores.

Results

Broad-scale study

No evidence of linkage disequilibrium was detected between any of the eight nuclear microsatellites analysed. Between nine (FR646655) and 51 (M230) alleles were detected per locus, with a total of 261 (mean = 32.625 per locus; Table 2). Levels of observed (H_O) and expected (H_E) heterozygosity ranged from 0.442 (FR646655) to 0.909 (M230; mean = 0.709), and from 0.477 (FR646655) to 0.937 (M230; mean = 0.765), respectively. Levels of F_{IS} ranged from -0.004 (Femsatl-16) to 0.236 (Femsatl-8), with a mean value of 0.067. The estimated frequency of null alleles ranged from zero (Femsatl-16) to 0.142 (Femsatl-8), with a mean value of 0.041. The proportion of large alleles not called by the GENEMAPPER software under the default settings in the four loci where there was significant large allele dropout (Femsatl-4, Femsatl-8, Femsatl-11 and M230) ranged from 2.98% (Femsatl-4) to 11.59% (M230).

Within populations, levels of allelic richness (A_R) averaged over loci ranged from 9.52 (Glenarm Forest) to 11.52 (Killeter Forest), with a mean value of 10.53 (Table 1). A total of 41 private alleles was detected, with the number per population ranging from zero to four. The majority (38) of these were restricted to a single individual, with the remaining three being found in two individuals. Levels of observed (H_O) and expected (H_E) heterozygosity ranged from 0.637 (Castle Hill) to 0.823 (Glenarm Forest; mean = 0.709), and from 0.712 (Trassey Road) to 0.809 (Rostrevor and Randalstown; mean = 0.765) respectively. The heterozygote deficit observed in the majority of the populations gave rise to F_{IS} values which were significantly higher than zero in 27 of the 36 populations studied, ranging from 0.053 (Killeter Forest) to 0.168 (Letterfrack; mean = 0.067). Diversity statistics for individual loci by population are given in Table S3, Supporting Information, and indicate that significant F_{IS}

values were generally due to high values at locus Femsatl-8, which were significantly higher than zero in 32 of the 36 population studied, suggesting the presence of null alleles at this locus.

Five of the ten chloroplast microsatellite loci studied were polymorphic in the samples analysed, exhibiting between two and four alleles (Table S1, Supporting Information). Combining allele sizes across loci gave eight haplotypes (See Figure S1, Supporting Information for a network of evolutionary relationships between haplotypes). One of these (H1) was found in the vast majority (995 out of 1052) of the trees studied. Levels of haplotype diversity (H) ranged from zero (several populations) to 0.572 (Barnett Demesne; Table 1).

Levels of population differentiation were $\Phi_{ST} = 0.0131$ and Hedrick's $G'_{ST} = 0.0547$ for the nuclear microsatellites, and $\Phi_{ST} = 0.2629$ for the chloroplast microsatellites (results of the AMOVA are given in Table 3). The BAPS analysis assigned all 36 populations to a single genetic cluster, although a weak but significant isolation-by-distance ($P = 0.005$) was observed across all populations, but not across NI populations only ($P = 0.09$; Figure 3). Finally, the spatial autocorrelation analyses revealed very little significant within-population spatial genetic structuring, with structuring only observed up to 50 m in the Dromora, Rostrevor, Randalstown and Lemnagore Wood populations (Figure S2, Supporting Information), and Sp values ranging from 0.000 (several populations) to 0.020 (Knockninney Hill; Table 1).

Fine-scale study

We successfully genotyped 140 adult trees and 93 saplings from Barnett Demesne, and 44 adults and 39 saplings from Cregagh Glen. For the Barnett Demesne stand, there was extremely good agreement between parentage based on strict exclusion, and assignments

266 based on likelihood implemented in CERVUS: in only five cases, CERVUS identified a second
267 parent where strict exclusion only identified a single parent, and there were four cases where
268 a sapling/parent combination was identified by exclusion, but where the adult had a LOD
269 score below the threshold calculated by CERVUS. Based on the CERVUS results, a single
270 parent was identified for 42 saplings, both parents were identified for 41 saplings, and no
271 parent within the stand was identified for five saplings. Three putative parents above the
272 LOD threshold were identified for a single sapling, which was not included further in the
273 analysis, as were the four saplings where a parent was identified by exclusion, but this adult
274 had a LOD score below the threshold. Three chloroplast microsatellite haplotypes were
275 identified, and in the 41 cases where both parents were identified, differences in chloroplast
276 haplotypes between the parents allowed the identification of the seed parent in 13 cases.
277 Consequently, seed dispersal distances could be calculated for 55 of the saplings: 13 where
278 the seed parent was identified in the parent-pair, and for the 42 saplings where a single parent
279 was identified, since the assumption that a single parent is the seed parent is far more
280 parsimonious than the alternative explanation of the adult being the pollen parent, which
281 pollinated another tree outside the stand, with the seed subsequently dispersing back into the
282 stand. Furthermore, in all cases of single parent assignment, there was a match between the
283 adult and sapling chloroplast haplotype, consistent with the adult being the seed parent. This
284 includes the rarest haplotype, found in a single sapling and a single adult, which were classed
285 as parent-offspring pairs by both CERVUS and strict exclusion. Dispersal distances ranged
286 from 3 to 223 m (mean = 42 m; median = 31 m; Figure 4). Pollination distances were
287 calculated for the 13 parent-pairs, and ranged from 2 to 266 m (mean = 93 m; median = 83
288 m). Realized pollen dispersal distances i.e. from pollen parent to sapling ranged from 7 to
289 168 m (mean = 65 m; median = 47 m; Table 4).

290 In the Cregagh Glen stand, very few putative parents were identified by CERVUS which
291 had LOD scores above the critical value. This was due to a combination of lower overall
292 genetic variation, and the occurrence of high-frequency alleles at several of the microsatellite
293 loci. Four dispersal events from separate single parents were identified, with distances of 23,
294 82, 123 and 148 m. In eleven cases, however, no parent was identified, suggesting
295 immigration of seed into the stand. All adults and saplings shared a single chloroplast
296 microsatellite haplotype.

Discussion

Lack of genetic structure and implications for restorative planting

For over 50 years now, the concept of provenance has been integral to forestry practices, particularly with respect to restocking and / or replanting of woodlands (reviewed in Jones and Burley 1973). This reflects observed phenotypic and underlying genetic variation across species' distributions, and recommends that where possible, woodlands should be restocked with local seeds or seedlings. Our finding that ash woodlands across Ireland are genetically uniform suggests that the concept of provenance might more usefully reflect the geographic distribution of genetic variation, and that all could be treated as a single management unit, given the lack of genetic differentiation between populations and the low incidence of private alleles. The observed level of population differentiation based on nuclear microsatellites ($\Phi_{ST} = 0.0131$) was the second lowest reported for European ash, with previous studies estimating between 1.2% (Hebel et al. 2006) and 8.7% (Heuertz et al. 2001) of nuclear diversity partitioned between populations, and is consistent with wind pollination and seed dispersal (Wardle 1961). Unfortunately, these previous studies did not calculate comparable statistics to the G'_{ST} value of 0.0547 observed in the present study, but future studies using microsatellites should calculate the equivalent statistic to take into account underestimation of levels of differentiation when using highly variable markers (Hedrick 2005).

Replacement of native trees for whatever reason should be based on knowledge of the geographic distribution of genetic variation (Godefroid et al. 2011). Our results clearly indicate that the source of material for replanting ash, at least in Ireland, is largely irrelevant, given such low levels of differentiation. The inclusion of samples from the extreme east (Co. Wicklow), south (Co. Cork) and west (Co. Galway) of Ireland in the same genetic cluster as the 33 populations from Northern Ireland indicates that our findings are probably applicable

to ash woodlands across the island of Ireland as a whole. Furthermore, the Irish samples of ash exhibited similar levels of nuclear genetic diversity to those in Great Britain and continental Europe, including populations from putative refugial areas that should harbour the highest levels of variation (Heuertz et al. 2001; Morand et al. 2002; Heuertz et al. 2004a; Heuertz et al. 2004b; Ferrazzini et al. 2007; Sutherland et al. 2010; Gérard et al. 2013). However, such replanting should be carried out using native material from long-established, unplanted / unstocked woodlands, since recent studies have shown that material used for replanting in Ireland often contains individuals that possess alleles introgressed from the congeneric *F. angustifolia* (Thomasset et al. 2013).

Levels of chloroplast genetic diversity were very low, with a single haplotype found in almost 95% of all the individuals studied. This haplotype most likely corresponds to Haplotype H04 from Heuertz et al. (2004a), which is also the dominant haplotype in Britain as a result of postglacial recolonization from Iberia. Populations in the east of Northern Ireland tended to exhibit the highest levels of chloroplast diversity, with most of the populations in the west fixed for the most common haplotype. This could be due to founder effects associated with westward recolonization from Britain or to predominantly eastward seed dispersal by prevailing westerly winds, since the chloroplast genome is maternally inherited in ash, or to a combination of both.

Several previous population genetic studies on ash have reported significant, and often high, levels of F_{IS} , which have been attributed to various factors including inbreeding, null alleles, large allele dropout and Wahlund effect (Morand et al. 2002; Ferrazzini et al. 2007; Sutherland et al. 2010). The mean F_{IS} calculated for ash populations across Northern Ireland (0.067) is significantly lower than that reported by Sutherland et al. (2010), who used six of the eight loci analysed in the present study to examine populations throughout Great Britain (mean F_{IS} = 0.182; Mann-Whitney test, $z = 6.07$, $P < 0.0001$). We designed new primers to

amplify previously characterized microsatellite loci with the aim of circumventing any potential problems with null alleles, and our estimated null allele frequencies were generally much lower than those reported in Sutherland et al. (2010). However, on genotyping a subset of our samples using the same primers as Sutherland et al. (2010), we did not observe an increase in F_{IS} or estimated null allele frequencies, suggesting that the previously observed homozygote deficiencies were not due to null alleles as a result of non-amplification. Furthermore, although Femsatl-8 exhibited the highest F_{IS} among the loci analysed in both cases, which might suggest null alleles associated with this particular marker, the same locus exhibited the lowest F_{IS} in a previous study in Germany (Hebel et al. 2006). The fact that this locus was significantly higher than zero in 32 of the 36 populations studied, even where the majority of the other alleles in these populations did not yield significant F_{IS} values, however, does suggest the presence of null alleles.

Another potential cause of heterozygote deficiency is large allele dropout, where short alleles are preferentially amplified during the PCR. Automated scoring software packages, such as GENOTYPER and GENEMAPPER, will often not identify these long alleles. We took the precaution of manually checking each chromatogram, and using the default settings for allele scoring in the GENEMAPPER package, we identified uncalled large alleles at frequencies ranging from 2.98% to 11.59% at four of the eight loci studied (see Figure S3, Supplementary information for an example). The phenomenon is particularly prevalent at loci with a high number of alleles over a large size range, and with such high frequencies of uncalled alleles, analyses based on the raw outputs from these genotyping packages would result in apparent homozygote excesses and subsequently inflated F_{IS} values.

Sutherland et al. (2010) suggested that the F_{IS} values observed in their study might be due to a Wahlund effect, namely the occurrence of spatial genetic structuring within populations, a scenario also proposed to a lesser degree by Ferrazzini et al. (2007). Because we recorded

GPS coordinates for each of the trees sampled in the present study, we were able to carry out spatial autocorrelation analyses to test for such structuring. With the exception of significantly higher levels of relatedness up to 50 m in only four populations out of the 36 studied, we found no evidence of Wahlund effects.

Given that we can exclude null alleles (with the possible exception of locus Femsat1-8, which consistently exhibits high F_{IS} levels across most studies), large allele dropout and Wahlund effects, the F_{IS} values observed would appear to give a true measure of the levels of inbreeding in populations of ash in the present study. Our spatial autocorrelation analyses found little evidence for the breeding “subunits” previously suggested to exist within ash woodlands (Heuertz et al. 2001; Morand et al. 2002). The mean value of S_p calculated for the 36 populations studied (0.006) was lower than the mean value reported for trees (0.010) by Vekemans and Hardy (2004) and the mean value from six microsatellite-based studies in tropical trees (0.017; Hardy et al. 2006), although higher than that calculated for a Romanian population of *F. excelsior* (0.002; Heuertz et al. 2003). This may be due at least in part to the differing densities of ash trees in the various woodlands analysed in the present study. For example, the Knockninney Hill population, which presented the highest S_p value, had only a single pair of individuals within 100 m of each other.

Evidence for frequent long-distance dispersal events

Although the use of molecular genetic techniques, particularly high-resolution microsatellite markers, has provided valuable insights into seed dispersal in natural plant populations (Powell et al. 1996; Ashley 2010), there still remain problems associated with identifying the seed parents of established plants and / or seedlings in monoecious species. Estimates of pollen-mediated gene flow can be relatively easily obtained by genotyping seeds and “subtracting” the genotype of the maternal plant, thus leaving the paternal genotype which

can be matched to putative parent plants. Such an approach has been employed previously in ash, indicating pollination events at distances over several hundred metres, and up to nearly 3 km (Bacles et al. 2005; Bacles and Ennos 2008; Thomasset et al. 2014). Estimating seed dispersal, however, is a more difficult process, particularly in monoecious species (Sork and Smouse 2006). Previous studies have attempted to genotype the endocarp tissue to identify the maternal parent of dispersed seeds (Godoy and Jordano 2001; Garcia et al. 2007), but this only provides estimates of initial dispersal, and does not necessarily provide an indication of true population demography and recruitment (effective dispersal), for which identification of the mothers of seedlings or established plants is necessary. For seedlings in a population of an outcrossing species, it may be possible to identify both parents, one parent, which will be either the pollen or seed parent, or neither parent, indicating immigration of seed into the population. In angiosperms, chloroplast-specific markers can be used in conjunction with biparentally inherited nuclear markers to assign the maternal and the paternal parent where both parents are identified, since the chloroplast genome is usually maternally inherited. One drawback of such an approach is the low mutation rate in chloroplast genomes, meaning that often there is inadequate resolution to assign the maternal parent (Wolfe et al. 1987). By using highly polymorphic chloroplast microsatellite markers, which allow the high-resolution of maternal genotypes, it may often be possible to determine which is the mother plant in cases where both parents are identified using nuclear microsatellites (Provan et al. 2001).

By using a combination of nuclear and chloroplast microsatellite markers, we were able to assign seed and pollen parents unambiguously for 13 out of 41 saplings for which both parents were identified within the Barnett Demesne stand, as well as assigning putative seed parents to a further 42 saplings. Previous studies using genetic markers to identify the source of established seedlings relied on genotyping any maternal tissue associated with the seedling, but these approaches can be problematic due to the low quality of DNA typically

recovered from the pericarp (Grivet et al. 2009; Smouse et al. 2012). Chloroplast microsatellite markers provide a convenient, high-resolution, uniparental assay (maternal in the majority of angiosperms and paternal in the majority of gymnosperms) that can be run on leaf material from established plants, and thus allow the assignment of seed and pollen parents where both parents are identified (Provan et al. 2001; Ebert and Peakall 2009a), circumventing the need to rely on genotypes from maternal tissues. Primers to amplify chloroplast microsatellites are available for a wide range of species, and the high levels of conservation of the chloroplast genome means that primers developed for a particular species often give polymorphic markers in related taxa (Provan et al. 2001). In addition, sets of universal primers are available to facilitate *de novo* development of these markers, particularly for non-model organisms or taxa with little sequence information available in DNA sequence databases such as GenBank (e.g. Ebert and Peakall 2009b).

Our findings indicate frequent seed dispersal over distances greater than 100 m, with six known within-stand dispersal events (over 10 %) exceeding this range. We also identified immigration into the stand in 16 cases (five out of 93 [5%] from Barnett Demesne and eleven out of 39 [28%] from Cregagh Glen). Barnett Demesne is located in a largely urban area, and the nearest stand of ash trees was 400 m to the east, at Shaw's bridge (Fig. 1), suggesting that this was the minimum dispersal distance of immigrant seed into the stand. Despite the lower genetic diversity in the Cregagh Glen population, which led to a lower level of parentage assignment, the higher rate of immigration appeared to result from extra-stand fertilization. Cregagh Glen is in a more agriculture-dominated landscape on the eastern edge of Belfast, and it is possible that immigrant seed dispersed from neighbouring isolated individuals, low-density hedgerow trees, or from the next-nearest substantial stand of ash, which was a similarly-sized stand in a ravine *ca.* 500 m to the east (Fig. 1).

Only two previous genetic studies on seed dispersal in ash have been carried out. Heuertz et al. (2003) used simulation studies coupled with estimates of kinship from five biparentally inherited nuclear microsatellites, including four of the seven loci used in the present study, to infer levels of seed and pollen dispersal in a continuous forest in southeast Romania. The estimated levels of seed dispersal, which were ≤ 14 m, were lower than both the mean and median values calculated for the Barnett Demesne stand in the present study, and far lower than the majority of individual events identified. Bacles et al. (2006) used a direct, individual-based approach to assess seed dispersal in a highly fragmented landscape in southern Scotland. They detected multiple long-distance events, often between fragments of up to 1.4 km, but this is most likely due to the chronically fragmented nature of their study site, with far fewer barriers to dispersal, and the increased chance of the winged seeds being uplifted in the initial stages of dispersal. This scenario is very different to the closed, semi-continuous woodlands analysed in the present study, and our findings may better reflect patterns of dispersal in more typical mixed-deciduous woodlands. Interestingly, since Bacles et al. (2006) had no means to identify the seed parent where parent pairs were identified, they assumed that the closer of the two to the seedling was the seed parent, but our analysis indicated that the seed parent was the more distant parent of the two in four out of twelve cases (and in one case they were equidistant). This further highlights the utility and importance of our approach in unambiguously identifying maternal and paternal parents to accurately quantify dispersal.

The ability to identify the seed sources of established plants allows insights into the end-results of dispersal in population demography i.e. initial dispersal followed by germination and survival / recruitment into the population. This means that post-dispersal processes, such as competition and density-dependent mortality, can be addressed. This was not possible in early genetic studies on dispersal, which relied on genotyping seeds, and thus could only

471 assess initial seed dispersal (e.g. Godoy and Jordano 2001; Ziegenhagen et al. 2003; Grivet et
472 al. 2005). Although we did not specifically test for such effects, our plot of effective seed
473 dispersal distances within Barnett Demesne is consistent with a Janzen-Connell recruitment
474 process (Janzen 1970; Connell 1971; Augspurger 1983). Dispersal in the stand peaked at 30
475 – 40 m, before tailing off quickly, suggesting density-dependent mortality close to the mother
476 plant. A similar pattern was observed in a genetic study on Aleppo pine (*Pinus halepensis*)
477 specifically designed to test for Janzen-Connell effects (Steinitz et al. 2011).

Conclusions

Our results suggest that although there is considerable genetic variation in ash trees across the whole of Ireland, there is no evidence of population genetic structure. Hence, the imposition of “seed zones” as part of a recovery plan for ash trees in the aftermath of near total mortality due to ash dieback may not be justified, and is an avoidable cost. Our findings of frequent, long-distance dispersal events have further implications for the survival and persistence of ash woodlands in the face of a range of threats. Infection by the causal agent of ash dieback, *Chalara fraxinea*, may lead to loss of woodlands, either by pathogenic mortality or by anthropogenic clearance as a means of control (Pautasso et al. 2013). The high capacity for dispersal indicated by our results suggests good potential for natural regeneration, as well as for the spread of resistance to the disease, both via seeds and via pollen-mediated gene flow from individuals exhibiting inherent resistance. In addition, high levels of migration will be necessary to respond to global climate change, although this is very much dependent on the rate and extent of these changes.

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501 **Data archiving statement**

502

503 All data will be deposited in DRYAD on acceptance.

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Table 1 Details of populations studied. N – number of individuals analysed; A_R – allelic richness; P – number of private alleles; H_O – observed heterozygosity; H_E – expected heterozygosity; F_{IS} – inbreeding coefficient; H1-H8 – frequency of chloroplast haplotypes; H – gene diversity.

No	Name	Lat (N)	Long (W)	Nuclear							Chloroplast									
				N	A_R	P	H_O	H_E	F_{IS}	Sp^a	N	H1	H2	H3	H4	H5	H6	H7	H8	H
1	Portaferry	54.391	5.565	30	9.80	-	0.673	0.750	0.104***	0.000	30	28	2	-	-	-	-	-	-	0.129
2	Downpatrick	54.352	5.700	30	10.16	-	0.647	0.734	0.121***	0.003	30	30	-	-	-	-	-	-	-	0.000
3	Helen's Bay	54.672	5.731	30	10.17	2	0.656	0.730	0.103***	0.005	29	26	3	-	-	-	-	-	-	0.192
4	Magheramourne	54.810	5.781	30	10.24	-	0.782	0.784	0.003 ^{NS}	0.001	30	30	-	-	-	-	-	-	-	0.000
5	Glenarm Forest	54.962	5.958	30	9.52	2	0.823	0.770	-0.070 ^{NS}	0.008	30	29	-	1	-	-	-	-	-	0.067
6	Barnett Demesne	54.552	5.960	29	10.11	-	0.733	0.759	0.034 ^{NS}	0.001	30	12	16	-	-	1	1	-	-	0.572
7	Trassey Road	54.219	5.984	29	10.14	-	0.662	0.712	0.072**	0.010	26	26	-	-	-	-	-	-	-	0.000
8	Dromara	54.330	5.996	29	11.42	1	0.723	0.782	0.078**	0.011	29	29	-	-	-	-	-	-	-	0.000
9	Hillsborough	54.459	6.083	30	10.80	-	0.680	0.780	0.130***	0.007	28	19	9	-	-	-	-	-	-	0.452
10	Glenariff Forest	55.016	6.100	30	10.85	-	0.727	0.775	0.063*	0.010	29	28	-	-	-	-	-	1	-	0.069
11	Rostrevor	54.095	6.191	30	10.54	1	0.731	0.809	0.016 ^{NS}	0.013	26	26	-	-	-	-	-	-	-	0.000
12	Ballycastle Forest	55.174	6.226	30	9.82	1	0.755	0.757	0.003 ^{NS}	0.016	28	28	-	-	-	-	-	-	-	0.000
13	Randalstown	54.733	6.320	30	11.39	2	0.731	0.809	0.097***	0.015	28	28	-	-	-	-	-	-	-	0.000
14	Portglenone	54.863	6.472	30	10.41	3	0.739	0.802	0.080**	0.000	30	28	1	-	-	-	-	-	1	0.131
15	Gosford Park	54.303	6.522	30	11.07	2	0.738	0.771	0.044 ^{NS}	0.007	30	29	1	-	-	-	-	-	-	0.067
16	Ballymoney	55.062	6.560	30	9.90	1	0.697	0.771	0.098***	0.000	28	28	-	-	-	-	-	-	-	0.000
17	Peatlands Park	54.486	6.616	29	10.42	3	0.667	0.730	0.086**	0.012	29	26	-	-	3	-	-	-	-	0.192
18	Carndaisy Woods	54.749	6.725	30	10.31	1	0.690	0.774	0.110***	0.000	30	30	-	-	-	-	-	-	-	0.000
19	Downhill	55.160	6.807	29	10.33	1	0.656	0.765	0.145***	0.007	27	27	-	-	-	-	-	-	-	0.000
20	Drum Manor	54.639	6.815	30	11.34	3	0.727	0.779	0.068**	0.003	30	29	1	-	-	-	-	-	-	0.067

Table 1 (Continued)

No	Name	Lat	Long	Nuclear							Chloroplast									
		(N)	(W)	<i>N</i>	<i>A_R</i>	<i>P</i>	<i>H_O</i>	<i>H_E</i>	<i>F_{IS}</i>	<i>Sp^a</i>	<i>N</i>	H1	H2	H3	H4	H5	H6	H7	H8	<i>H</i>
21	Lemnagore Wood	54.331	6.841	29	10.18	-	0.714	0.720	0.008 ^{NS}	0.012	30	30	-	-	-	-	-	-	-	0.000
22	Roe Valley	55.025	6.938	30	9.93	-	0.669	0.777	0.141 ^{***}	0.015	30	29	-	1	-	-	-	-	-	0.067
23	Knockmany Forest	54.436	7.170	30	10.72	1	0.800	0.782	-0.023 ^{NS}	0.003	30	30	-	-	-	-	-	-	-	0.000
24	Slieve Beagh	54.380	7.203	28	10.92	-	0.670	0.733	0.088 ^{**}	0.006	30	28	-	2	-	-	-	-	-	0.129
25	Stranbane Glen	54.836	7.443	30	10.99	4	0.708	0.773	0.084 ^{***}	0.002	30	30	-	-	-	-	-	-	-	0.000
26	Crom	54.170	7.451	30	10.32	1	0.669	0.755	0.115 ^{***}	0.009	30	30	-	-	-	-	-	-	-	0.000
27	Knockninny Hill	54.231	7.573	28	11.15	3	0.691	0.770	0.103 ^{***}	0.020	30	30	-	-	-	-	-	-	-	0.000
28	Sloughan Glen	54.615	7.574	29	9.82	-	0.697	0.745	0.065 [*]	0.004	30	30	-	-	-	-	-	-	-	0.000
29	Castle Hill	54.484	7.722	30	11.14	1	0.637	0.757	0.161 ^{***}	0.000	30	30	-	-	-	-	-	-	-	0.000
30	Ely Lodge	54.412	7.725	30	11.39	3	0.728	0.776	0.062 ^{**}	0.011	30	30	-	-	-	-	-	-	-	0.000
31	Killeter Forest	54.687	7.744	30	11.52	1	0.738	0.779	0.053 [*]	0.001	30	30	-	-	-	-	-	-	-	0.000
32	Marble Arch	54.267	7.810	28	10.24	1	0.710	0.763	0.071 ^{**}	0.008	30	30	-	-	-	-	-	-	-	0.000
33	Castle Caldwell	54.493	7.965	30	11.21	2	0.687	0.742	0.075 ^{**}	0.000	30	30	-	-	-	-	-	-	-	0.000
34	Glenasmole Valley	53.251	6.371	28	11.00	1	0.762	0.776	0.018 ^{NS}	0.007	30	19	11	-	-	-	-	-	-	0.481
35	Knocknamallavoge	51.853	8.527	29	9.63	-	0.746	0.802	0.071 [*]	0.000	29	29	-	-	-	-	-	-	-	0.000
36	Letterfrack	53.553	9.948	26	10.02	-	0.644	0.771	0.168 ^{***}	0.005	26	24	2	-	-	-	-	-	-	0.148

^a *Sp* is a measure of spatial genetic structure proposed by Vekemans and Hardy (2004). See Materials and Methods for details.

Table 2 Nuclear microsatellite loci analyzed in this study. N – number of alleles; H_O – observed heterozygosity; H_E – expected heterozygosity; F_{IS} – inbreeding coefficient; Null – null allele frequency; LAD – large allele dropout.

Locus	Primers*	N	Range (bp)	H_O	H_E	F_{IS}	Null	%LAD
FR639485	TGACAAACCCCAGCCTAACTCT GCCTGAGCAAGTAAAGACGCTA	21	310-348	0.613	0.629	0.024	0.019	-
FR646655	TGGAGCAGTTGAAGCACTGAAA TCTTCATCTTCCCAACAGCAGC	9	200-230	0.442	0.477	0.075	0.040	-
Femsatl-4	TTCATGCTTCTCCGTGTCTCAG GGGTGAAGAGGCTTTGTGTCAT	48	134-220	0.851	0.877	0.030	0.017	2.98
Femsatl-8	TTGCCTTTGTAGCTCAGG GCGTTGTCCTTAACTTTTCA	32	165-229	0.695	0.910	0.236	0.142	3.38
Femsatl-11	TGAACACAGCTCTTGACTCTGA GTTCTACTACTTCAAGAACAGGGGG	38	188-264	0.858	0.884	0.029	0.019	6.95
Femsatl-16	TGATCTCGTCCGAATTCAGTGC ATGATGGCGACTTTTGGTGTGA	13	193-225	0.500	0.499	-0.004	N/A	-
Femsatl-19 [†]	TCAAATTCCTGATTTTCAGGGGGA CGCGTATGATGGTCTTTATCTCTGT	49	137-217	0.801	0.905	0.116	0.068	-
M230	ACGCGCACGTTCTTTCTATTTG GCTTTCTTGACCGGCTGACTAT	51	214-328	0.909	0.937	0.030	0.019	11.59

* Forward tailed with CACGACGTTGTAAAACGAC; Reverse tailed with GTGTCTT

[†] Not used in fine-scale study

Table 3 Analysis of molecular variance (AMOVA).

Markers	Source of variation	Sum of squares	Variance	% variation
Nuclear	Among populations	183.471	0.03917	1.31
	Within populations	6114.600	2.93971	98.69
Chloroplast	Among populations	15.373	0.01370	26.29
	Within populations	39.066	0.03841	73.71

Table 4 Pollination distances and realized pollen dispersal distances i.e. from father to sapling for the 13 saplings where the maternal parent was identified in the parent-pair.

Sapling	Father	Mother	Distance (m)	
			Pollination	Realized
BS-03	BA-006	BA-056	83	67
BS-08	BA-001	BA-009	2	8
BS-10	BA-005	BA-015	22	9
BS-18	BA-083	BA-054	158	149
BS-34	BA-105	BA-026	190	168
BS-50	BA-033	BA-017	12	7
BS-52	BA-032	BA-081	167	23
BS-55	BA-076	BA-025	68	100
BS-62	BA-135	BA-068	108	135
BS-65	BA-037	BA-006	22	41
BS-70	BA-076	BA-092	266	47
BS-72	BA-109	BA-116	2	75
BS-83	BA-135	BA-074	112	13

Table S1 Coordinates for samples analysed in the present study

Location	Sample	Lat	Long
Barnett Demesne (Adults)	BA-001	54.55184233	-5.961468454
	BA-002	54.55175997	-5.961379820
	BA-003	54.55189750	-5.961542988
	BA-004	54.55175360	-5.961534711
	BA-005	54.55183309	-5.961453458
	BA-006	54.55174566	-5.961596936
	BA-007	54.55175151	-5.961411156
	BA-008	54.55181722	-5.961577908
	BA-009	54.55183309	-5.961453458
	BA-010	54.55162413	-5.960783766
	BA-011	54.55179692	-5.961439805
	BA-012	54.55170506	-5.961320730
	BA-013	54.55171378	-5.961304838
	BA-014	54.55163402	-5.961370647
	BA-015	54.55165146	-5.961338862
	BA-016	54.55167813	-5.961322074
	BA-018	54.55176764	-5.961302150
	BA-019	54.55178326	-5.961162256
	BA-020	54.55186562	-5.961250890
	BA-021	54.55166655	-5.961168080
	BA-022	54.55192624	-5.960583199
	BA-023	54.55163064	-5.961169871
	BA-024	54.55162088	-5.961123987
	BA-025	54.55163220	-5.961262537
	BA-026	54.55156988	-5.961296561
	BA-027	54.55164222	-5.961323866
	BA-028	54.55161399	-5.961247989
	BA-029	54.55161477	-5.961294321
	BA-030	54.55156936	-5.961265673
	BA-031	54.55157066	-5.961342894
	BA-032	54.55164300	-5.961370199
	BA-033	54.55164326	-5.961385643
	BA-034	54.55161581	-5.961356098
	BA-035	54.55158094	-5.961419667
	BA-036	54.55178091	-5.961023257
	BA-037	54.55189658	-5.960955656
	BA-038	54.55170206	-5.960609846
	BA-039	54.55179743	-5.960404141
	BA-040	54.55149572	-5.961161147
	BA-041	54.55205048	-5.959958702
	BA-042	54.55192233	-5.960351534
	BA-043	54.55188486	-5.960260661
	BA-044	54.55184921	-5.960277898
	BA-045	54.55195537	-5.960179854
	BA-046	54.55185923	-5.960339227
	BA-047	54.55188642	-5.960353327
	BA-048	54.55178935	-5.959925369
	BA-049	54.55206348	-5.959664362
	BA-050	54.55209380	-5.959863795

Table S1 (continued)

Location	Sample	Lat	Long
Barnett Demesne (Adults)	BA-051	54.551708	-5.961574
	BA-052	54.55170531	-5.960269625
	BA-053	54.55199792	-5.960038614
	BA-054	54.55188642	-5.960353327
	BA-055	54.55194405	-5.960041303
	BA-056	54.55192233	-5.960351534
	BA-057	54.55197840	-5.959946844
	BA-058	54.55188642	-5.960353327
	BA-059	54.55189436	-5.960291101
	BA-060	54.55186820	-5.960338779
	BA-061	54.55170479	-5.960238736
	BA-062	54.55194535	-5.960118525
	BA-063	54.55178037	-5.959925817
	BA-064	54.55197203	-5.960101736
	BA-065	54.55165	-5.960217
	BA-066	54.550867	-5.960833
	BA-067	54.55165	-5.960233
	BA-068	54.5515	-5.960617
	BA-069	54.5516	-5.96065
	BA-070	54.551883	-5.961233
	BA-071	54.550917	-5.960883
	BA-072	54.551	-5.96145
	BA-073	54.551217	-5.96175
	BA-074	54.550633	-5.9616
	BA-075	54.5516	-5.9608
	BA-076	54.551033	-5.9615
	BA-077	54.5519	-5.96125
	BA-078	54.551783	-5.95995
	BA-079	54.5518	-5.959883
	BA-080	54.551983	-5.958883
	BA-081	54.55205	-5.958883
	BA-082	54.55425	-5.960983
	BA-083	54.5531	-5.96165
	BA-084	54.55268	-5.9602
	BA-085	54.55295	-5.961483
	BA-086	54.5541	-5.961
	BA-087	54.55417	-5.9612
	BA-088	54.554	-5.961867
	BA-089	54.55312	-5.9617
	BA-090	54.55293	-5.9613
	BA-091	54.55465	-5.957533
	BA-092	54.55212	-5.957833
	BA-093	54.55328	-5.957567
	BA-094	54.55662	-5.956933
	BA-095	54.55615	-5.95775
	BA-096	54.55648	-5.95715
	BA-097	54.55482	-5.959767
	BA-098	54.5531	-5.961717
	BA-099	54.55355	-5.962033
	BA-100	54.55255	-5.958633

Table S1 (continued)

Location	Sample	Lat	Long
Barnett Demesne (Adults)	BA-101	54.55208	-5.9579
	BA-102	54.5532	-5.961767
	BA-103	54.5525	-5.957933
	BA-104	54.55667	-5.956883
	BA-105	54.55325	-5.96185
	BA-106	54.55333	-5.961967
	BA-107	54.55247	-5.959067
	BA-108	54.55075	-5.9604
	BA-109	54.55063	-5.960217
	BA-110	54.55063	-5.960367
	BA-111	54.55133	-5.961183
	BA-112	54.55077	-5.960083
	BA-113	54.55067	-5.960067
	BA-116	54.55063	-5.960183
	BA-117	54.55068	-5.959883
	BA-118	54.55068	-5.960067
	BA-120	54.55063	-5.959883
	BA-121	54.5507	-5.959917
	BA-122	54.55055	-5.959883
	BA-124	54.55068	-5.959633
	BA-125	54.55063	-5.959567
	BA-126	54.55055	-5.959483
	BA-128	54.55062	-5.959567
	BA-129	54.55057	-5.959483
	BA-130	54.55057	-5.959417
	BA-131	54.55055	-5.959467
	BA-132	54.55055	-5.959433
	BA-133	54.55068	-5.959733
	BA-134	54.55065	-5.960217
	BA-135	54.55063	-5.959867
	BA-136	54.5508	-5.960317
	BA-137	54.55085	-5.961217
	BA-139	54.55075	-5.96015
	BA-140	54.55125	-5.961083
Barnett Demesne (Saplings)	BS-01	54.551876	-5.960802
	BS-02	54.551827	-5.960557
	BS-03	54.551872	-5.960586
	BS-04	54.551873	-5.960601
	BS-05	54.551788	-5.960915
	BS-06	54.551726	-5.961405
	BS-07	54.55178	-5.961518
	BS-08	54.55178	-5.961518
	BS-09	54.551844	-5.961592
	BS-10	54.551772	-5.961549
	BS-11	54.551663	-5.961477
	BS-12	54.551849	-5.960773

Table S1 (continued)

Location	Sample	Lat	Long
Barnett Demesne (Saplings)	BS-13	54.551914	-5.960383
	BS-14	54.551716	-5.961428
	BS-15	54.551929	-5.960212
	BS-16	54.552011	-5.95912
	BS-18	54.55185	-5.960881
	BS-19	54.55194	-5.960335
	BS-20	54.551817	-5.961037
	BS-21	54.551844	-5.961051
	BS-22	54.551922	-5.960877
	BS-23	54.55194	-5.960892
	BS-24	54.551922	-5.960893
	BS-26	54.551835	-5.961021
	BS-27	54.551862	-5.961035
	BS-28	54.551879	-5.961003
	BS-29	54.551869	-5.960926
	BS-30	54.55177	-5.960916
	BS-31	54.551781	-5.961054
	BS-32	54.551798	-5.960961
	BS-33	54.551836	-5.961082
	BS-34	54.551817	-5.961037
	BS-35	54.551781	-5.961023
	BS-36	54.551772	-5.961039
	BS-37	54.55179	-5.961579
	BS-38	54.55177	-5.9609
	BS-39	54.551615	-5.96131
	BS-40	54.551642	-5.961308
	BS-41	54.551641	-5.961278
	BS-42	54.551794	-5.960729
	BS-43	54.552073	-5.95971
	BS-44	54.55181	-5.96062
	BS-45	54.55181	-5.960635
	BS-46	54.552033	-5.95999
	BS-47	54.551836	-5.960572
	BS-48	54.551897	-5.96043
	BS-49	54.551913	-5.960321
	BS-50	54.551698	-5.961445
	BS-51	54.551921	-5.960831
	BS-52	54.551839	-5.961268
	BS-53	54.551799	-5.961038
	BS-54	54.551817	-5.961006
	BS-55	54.551842	-5.960927
	BS-56	54.551771	-5.960977
	BS-57	54.551725	-5.960902
	BS-58	54.551779	-5.9609
	BS-59	54.551598	-5.961342
	BS-60	54.551852	-5.960463
	BS-61	54.551892	-5.960693
	BS-62	54.551672	-5.960951

Table S1 (continued)

Location	Sample	Lat	Long
Barnett Demesne (Saplings)	BS-63	54.551852	-5.960417
	BS-64	54.551617	-5.96145
	BS-65	54.55165	-5.961417
	BS-66	54.55193	-5.960815
	BS-67	54.551781	-5.961054
	BS-68	54.551167	-5.96095
	BS-69	54.55095	-5.9606
	BS-70	54.5511	-5.960783
	BS-71	54.551167	-5.960933
	BS-72	54.551167	-5.9609
	BS-73	54.551167	-5.960883
	BS-74	54.551067	-5.960933
	BS-75	54.551117	-5.960967
	BS-76	54.550733	-5.9596
	BS-77	54.55065	-5.9595
	BS-79	54.550733	-5.959633
	BS-80	54.5509	-5.960667
	BS-81	54.550717	-5.959633
	BS-82	54.550667	-5.959633
	BS-83	54.55065	-5.959667
	BS-84	54.5506	-5.959483
	BS-85	54.550567	-5.95955
	BS-86	54.550783	-5.960283
	BS-87	54.550883	-5.960683
	BS-88	54.551317	-5.961117
	BS-89	54.55135	-5.961167
	BS-90	54.551217	-5.961017
	BS-91	54.551333	-5.9611
	BS-92	54.551067	-5.960783
	BS-93	54.550967	-5.960717
	BS-94	54.550917	-5.960667
	BS-95	54.551117	-5.960967
	BS-96	54.550883	-5.960683
Cregagh Glen (Adults)	CA-01	54.56205	-5.88915
	CA-02	54.56205	-5.88905
	CA-03	54.56435	-5.88995
	CA-04	54.564267	-5.88978
	CA-05	54.563717	-5.8897
	CA-06	54.56515	-5.889833
	CA-07	54.563533	-5.889533
	CA-08	54.5632833	-5.88945
	CA-09	54.563033	-5.88941667
	CA-10	54.562333	-5.88933
	CA-11	54.564633	-5.8897167
	CA-12	54.564167	-5.8899667
	CA-13	54.565033	-5.8899167
	CA-14	54.5620833	-5.88905

Table S1 (continued)

Location	Sample	Lat	Long
Cregagh Glen (Adults)	CA-15	54.5637	-5.8896
	CA-16	54.5644833	-5.8897
	CA-17	54.5621667	-5.888733
	CA-18	54.5632167	-5.889433
	CA-19	54.5644167	-5.8897167
	CA-20	54.563733	-5.8898
	CA-21	54.5640833	-5.8898
	CA-22	54.5637667	-5.8895667
	CA-23	54.564133	-5.8894667
	CA-24	54.5678	-5.8914167
	CA-25	54.5666	-5.8909
	CA-26	54.5616	-5.8887333
	CA-27	54.5665833	-5.8910333
	CA-28	54.5665833	-5.89115
	CA-29	54.566633	-5.89115
	CA-30	54.565933	-5.8905833
	CA-31	54.5655833	-5.890416
	CA-32	54.567233	-5.89115
	CA-33	54.5668667	-5.8910167
	CA-34	54.562068	-5.888221
	CA-35	54.560998	-5.88841
	CA-36	54.5617167	-5.8885
	CA-37	54.56395	-5.8894167
	CA-38	54.5638	-5.88955
	CA-39	54.5649197	-5.88985
	CA-40	54.5641167	-5.889333
	CA-41	54.5663167	-5.890783
	CA-42	54.565033	-5.889797
	CA-43	54.565136	-5.889807
	CA-44	54.565233	-5.889806
Cregagh Glen (Saplings)	CS-01	54.565041	-5.889909
	CS-02	54.565037	-5.889909
	CS-03	54.565042	-5.889898
	CS-04	54.565037	-5.889885
	CS-05	54.565044	-5.889906
	CS-06	54.565044	-5.889908
	CS-07	54.565031	-5.889896
	CS-08	54.565033	-5.889889
	CS-10	54.565033	-5.889883
	CS-11	54.5643	-5.8898
	CS-12	54.565046	-5.889922
	CS-13	54.56429	-5.889808
	CS-14	54.565036	-5.889877
	CS-15	54.56429	-5.88979
	CS-16	54.564968	-5.890064
	CS-17	54.565041	-5.889916
	CS-18	54.565043	-5.889904

Table S1 (continued)

Location	Sample	Lat	Long
Cregagh Glen (Saplings)	CS-19	54.565045	-5.889924
	CS-20	54.565024	-5.889896
	CS-21	54.56502	-5.88989
	CS-22	54.565033	-5.8899
	CS-23	54.565033	-5.889867
	CS-24	54.565033	-5.889933
	CS-25	54.564	-5.88955
	CS-26	54.565	-5.8899167
	CS-27	54.5642	-5.88985
	CS-28	54.564	-5.88955
	CS-29	54.564	-5.889533
	CS-30	54.564	-5.8895667
	CS-31	54.564	-5.88955
	CS-32	54.56503	-5.8899
	CS-33	54.564	-5.88955
	CS-34	54.564	-5.889533
	CS-35	54.5636	-5.889533
	CS-36	54.565067	-5.8899
	CS-37	54.565033	-5.8899
	CS-38	54.564217	-5.88975
	CS-39	54.564	-5.88955
	CS-40	54.5637167	-5.8897

Table S2 Chloroplast microsatellite loci analyzed in this study.

Multiplex	Locus	Primers*	Alleles (bp)
1	AF528042.1	ACGAGCCAAAGTTCTAGCACAA GCCGGTTCGGGCTGATTTAT	181
	AM933080.1	ACATTCCTCCGCTTTCATTCCT TCTTCCTGCCACCTTTCCCA	125,127,128,129
	AF225238	GGGGGTAAAGACCACTCAATAAATGAA TCCTCGTACGGCTCGAGAAA	265
2	AF528042.2	ATGGATGGGGTGGGGTATTAGT CTCAAATCATATCAGAGGGGTTTGC	224
	JN590973	AGATAAAGGAAGGGGTCGAACG CAGGCCAGGCCATCAGAATAA	131,132
	AY911655	ACAGGAATCTTTCACAACTTCCCA CGAATTCCGCATATTTTCACATCTAGG	270,271
3	AF528042.3	GCTGGTTGCTTTTTCTTTCCCA CGTCTCAACGGAGAGTTCTGAGTC	184
	HM222783	CTTAGGGAAATCTCTTTCTACCG GTCAAGTCGATTTCAGATTATTCCAACG	121
	AM933080.2	GGATCAAGTACGGGTTTCCGAT ACTGGAACCCTTGAATTCATTAGATACT	122,123,124
4	FR639483	TGACAAACCCAGCCTAACTCT GCCTGAGCAAGTAAAGACGCTA	172,173,174

* Forward tailed with CACGACGTTGTAAAACGAC; Reverse tailed with GTGTCTT

Table S3 Diversity statistics for each locus by population

Population	Locus							
	FR639485	FR646655	Femsatl-4	Femsatl-8	Femsatl-11	Femsatl-16	Femsatl-19	M230
Portaferry	$H_O = 0.552$	$H_O = 0.367$	$H_O = 0.900$	$H_O = 0.600$	$H_O = 0.759$	$H_O = 0.448$	$H_O = 0.897$	$H_O = 0.862$
	$H_E = 0.645$	$H_E = 0.461$	$H_E = 0.880$	$H_E = 0.836$	$H_E = 0.893$	$H_E = 0.448$	$H_E = 0.908$	$H_E = 0.926$
	$F_{IS} = 0.147^{NS}$	$F_{IS} = 0.207^{NS}$	$F_{IS} = -0.024^{NS}$	$F_{IS} = 0.286^{***}$	$F_{IS} = 0.153^*$	$F_{IS} = 0.000^{NS}$	$F_{IS} = 0.013^{NS}$	$F_{IS} = 0.070^{NS}$
Downpatrick	$H_O = 0.428$	$H_O = 0.345$	$H_O = 0.759$	$H_O = 0.483$	$H_O = 0.897$	$H_O = 0.500$	$H_O = 0.862$	$H_O = 0.900$
	$H_E = 0.442$	$H_E = 0.448$	$H_E = 0.737$	$H_E = 0.912$	$H_E = 0.897$	$H_E = 0.590$	$H_E = 0.890$	$H_E = 0.958$
	$F_{IS} = 0.031^{NS}$	$F_{IS} = 0.234^{NS}$	$F_{IS} = -0.028^{NS}$	$F_{IS} = 0.475^{***}$	$F_{IS} = 0.000^{NS}$	$F_{IS} = 0.155^{NS}$	$F_{IS} = 0.032^{NS}$	$F_{IS} = 0.062^{NS}$
Helen's Bay	$H_O = 0.536$	$H_O = 0.379$	$H_O = 0.700$	$H_O = 0.593$	$H_O = 0.931$	$H_O = 0.433$	$H_O = 0.793$	$H_O = 0.884$
	$H_E = 0.544$	$H_E = 0.468$	$H_E = 0.843$	$H_E = 0.872$	$H_E = 0.859$	$H_E = 0.433$	$H_E = 0.930$	$H_E = 0.890$
	$F_{IS} = 0.016^{NS}$	$F_{IS} = 0.192^{NS}$	$F_{IS} = 0.172^*$	$F_{IS} = 0.325^{***}$	$F_{IS} = -0.085^{NS}$	$F_{IS} = 0.000^{NS}$	$F_{IS} = 0.150^{**}$	$F_{IS} = 0.006^{NS}$
Magheramourne	$H_O = 0.621$	$H_O = 0.500$	$H_O = 0.933$	$H_O = 0.767$	$H_O = 0.900$	$H_O = 0.633$	$H_O = 0.900$	$H_O = 1.000$
	$H_E = 0.623$	$H_E = 0.497$	$H_E = 0.870$	$H_E = 0.904$	$H_E = 0.896$	$H_E = 0.627$	$H_E = 0.905$	$H_E = 0.949$
	$F_{IS} = 0.003^{NS}$	$F_{IS} = -0.007^{NS}$	$F_{IS} = -0.074^{NS}$	$F_{IS} = 0.152^*$	$F_{IS} = -0.004^{NS}$	$F_{IS} = -0.010^{NS}$	$F_{IS} = 0.006^{NS}$	$F_{IS} = -0.055^{NS}$
Glenarm Forest	$H_O = 0.759$	$H_O = 0.467$	$H_O = 1.000$	$H_O = 0.897$	$H_O = 0.867$	$H_O = 0.733$	$H_O = 0.862$	$H_O = 1.000$
	$H_E = 0.734$	$H_E = 0.494$	$H_E = 0.828$	$H_E = 0.881$	$H_E = 0.830$	$H_E = 0.596$	$H_E = 0.899$	$H_E = 0.901$
	$F_{IS} = -0.034^{NS}$	$F_{IS} = 0.057^{NS}$	$F_{IS} = -0.213^{NS}$	$F_{IS} = -0.018^{NS}$	$F_{IS} = -0.045^{NS}$	$F_{IS} = -0.235^{NS}$	$F_{IS} = 0.042^{NS}$	$F_{IS} = -0.112^{NS}$
Barnett Demesne	$H_O = 0.679$	$H_O = 0.536$	$H_O = 0.929$	$H_O = 0.759$	$H_O = 0.793$	$H_O = 0.418$	$H_O = 0.793$	$H_O = 0.966$
	$H_E = 0.521$	$H_E = 0.529$	$H_E = 0.889$	$H_E = 0.919$	$H_E = 0.885$	$H_E = 0.497$	$H_E = 0.913$	$H_E = 0.917$
	$F_{IS} = -0.310^{NS}$	$F_{IS} = -0.013^{NS}$	$F_{IS} = -0.045^{NS}$	$F_{IS} = 0.177^*$	$F_{IS} = 0.106^{NS}$	$F_{IS} = 0.169^{NS}$	$F_{IS} = 0.133^*$	$F_{IS} = -0.054^{NS}$
Trassey Road	$H_O = 0.418$	$H_O = 0.345$	$H_O = 0.897$	$H_O = 0.750$	$H_O = 0.893$	$H_O = 0.310$	$H_O = 0.759$	$H_O = 0.931$
	$H_E = 0.492$	$H_E = 0.328$	$H_E = 0.898$	$H_E = 0.879$	$H_E = 0.917$	$H_E = 0.337$	$H_E = 0.901$	$H_E = 0.949$
	$F_{IS} = 0.162^{NS}$	$F_{IS} = -0.051^{NS}$	$F_{IS} = 0.001^{NS}$	$F_{IS} = 0.149^*$	$F_{IS} = 0.027^{NS}$	$F_{IS} = 0.080^{NS}$	$F_{IS} = 0.160^*$	$F_{IS} = 0.019^{NS}$

Table S3 (cont.)

Population	Locus							
	FR639485	FR646655	Femsatl-4	Femsatl-8	Femsatl-11	Femsatl-16	Femsatl-19	M230
Dromara	$H_O = 0.607$	$H_O = 0.429$	$H_O = 0.862$	$H_O = 0.793$	$H_O = 0.824$	$H_O = 0.536$	$H_O = 0.828$	$H_O = 0.897$
	$H_E = 0.609$	$H_E = 0.577$	$H_E = 0.873$	$H_E = 0.938$	$H_E = 0.897$	$H_E = 0.494$	$H_E = 0.913$	$H_E = 0.954$
	$F_{IS} = 0.003^{NS}$	$F_{IS} = 0.260^*$	$F_{IS} = 0.013^{NS}$	$F_{IS} = 0.157^{**}$	$F_{IS} = 0.084^{NS}$	$F_{IS} = -0.087^{NS}$	$F_{IS} = 0.096^{NS}$	$F_{IS} = 0.061^{NS}$
Hillsborough	$H_O = 0.433$	$H_O = 0.700$	$H_O = 0.679$	$H_O = 0.600$	$H_O = 0.900$	$H_O = 0.400$	$H_O = 0.769$	$H_O = 0.967$
	$H_E = 0.586$	$H_E = 0.677$	$H_E = 0.877$	$H_E = 0.929$	$H_E = 0.849$	$H_E = 0.458$	$H_E = 0.911$	$H_E = 0.949$
	$F_{IS} = 0.264^{NS}$	$F_{IS} = -0.034^{NS}$	$F_{IS} = 0.229^{**}$	$F_{IS} = 0.358^{***}$	$F_{IS} = -0.06^{NS}$	$F_{IS} = 0.128^{NS}$	$F_{IS} = 0.170^{**}$	$F_{IS} = -0.019^{NS}$
Glenariff Forest	$H_O = 0.655$	$H_O = 0.400$	$H_O = 0.893$	$H_O = 0.759$	$H_O = 0.900$	$H_O = 0.517$	$H_O = 0.759$	$H_O = 0.933$
	$H_E = 0.620$	$H_E = 0.513$	$H_E = 0.925$	$H_E = 0.895$	$H_E = 0.889$	$H_E = 0.514$	$H_E = 0.887$	$H_E = 0.954$
	$F_{IS} = -0.058^{NS}$	$F_{IS} = 0.223^*$	$F_{IS} = 0.035^{NS}$	$F_{IS} = 0.155^*$	$F_{IS} = -0.012^{NS}$	$F_{IS} = -0.006^{NS}$	$F_{IS} = 0.147^*$	$F_{IS} = 0.022^{NS}$
Rostrevor	$H_O = 0.571$	$H_O = 0.464$	$H_O = 0.893$	$H_O = 0.750$	$H_O = 0.964$	$H_O = 0.500$	$H_O = 0.786$	$H_O = 0.964$
	$H_E = 0.692$	$H_E = 0.384$	$H_E = 0.863$	$H_E = 0.865$	$H_E = 0.918$	$H_E = 0.444$	$H_E = 0.873$	$H_E = 0.952$
	$F_{IS} = 0.176^{NS}$	$F_{IS} = -0.215^{NS}$	$F_{IS} = -0.035^{NS}$	$F_{IS} = 0.135^{NS}$	$F_{IS} = -0.052^{NS}$	$F_{IS} = -0.130^{NS}$	$F_{IS} = 0.102^{NS}$	$F_{IS} = -0.013^{NS}$
Ballycastle Forest	$H_O = 0.533$	$H_O = 0.567$	$H_O = 0.933$	$H_O = 0.759$	$H_O = 0.963$	$H_O = 0.660$	$H_O = 0.900$	$H_O = 0.786$
	$H_E = 0.576$	$H_E = 0.485$	$H_E = 0.920$	$H_E = 0.874$	$H_E = 0.893$	$H_E = 0.544$	$H_E = 0.876$	$H_E = 0.892$
	$F_{IS} = 0.076^{NS}$	$F_{IS} = -0.171^{NS}$	$F_{IS} = -0.014^{NS}$	$F_{IS} = 0.134^*$	$F_{IS} = -0.080^{NS}$	$F_{IS} = -0.105^{NS}$	$F_{IS} = -0.028^{NS}$	$F_{IS} = 0.121^{NS}$
Randalstown	$H_O = 0.733$	$H_O = 0.552$	$H_O = 0.900$	$H_O = 0.733$	$H_O = 0.833$	$H_O = 0.500$	$H_O = 0.633$	$H_O = 0.967$
	$H_E = 0.669$	$H_E = 0.590$	$H_E = 0.912$	$H_E = 0.928$	$H_E = 0.911$	$H_E = 0.611$	$H_E = 0.898$	$H_E = 0.948$
	$F_{IS} = -0.097^{NS}$	$F_{IS} = 0.067^{NS}$	$F_{IS} = 0.014^{NS}$	$F_{IS} = 0.212^{***}$	$F_{IS} = 0.086^{NS}$	$F_{IS} = 0.185^{NS}$	$F_{IS} = 0.299^{***}$	$F_{IS} = -0.020^{NS}$
Portglenone	$H_O = 0.833$	$H_O = 0.500$	$H_O = 0.862$	$H_O = 0.633$	$H_O = 0.867$	$H_O = 0.586$	$H_O = 0.800$	$H_O = 0.828$
	$H_E = 0.758$	$H_E = 0.544$	$H_E = 0.858$	$H_E = 0.918$	$H_E = 0.891$	$H_E = 0.620$	$H_E = 0.914$	$H_E = 0.910$
	$F_{IS} = -0.101^{NS}$	$F_{IS} = 0.081^{NS}$	$F_{IS} = -0.004^{NS}$	$F_{IS} = 0.313^{***}$	$F_{IS} = 0.028^{NS}$	$F_{IS} = 0.056^{NS}$	$F_{IS} = 0.127^*$	$F_{IS} = 0.093^{NS}$

Table S3 (cont.)

Population	Locus							
	FR639485	FR646655	Femsatl-4	Femsatl-8	Femsatl-11	Femsatl-16	Femsatl-19	M230
Gosford Park	$H_O = 0.552$	$H_O = 0.517$	$H_O = 0.867$	$H_O = 0.633$	$H_O = 1.000$	$H_O = 0.567$	$H_O = 0.800$	$H_O = 0.967$
	$H_E = 0.606$	$H_E = 0.506$	$H_E = 0.912$	$H_E = 0.895$	$H_E = 0.913$	$H_E = 0.489$	$H_E = 0.894$	$H_E = 0.951$
	$F_{IS} = 0.090^{NS}$	$F_{IS} = -0.022^{NS}$	$F_{IS} = 0.051^{NS}$	$F_{IS} = 0.296^{***}$	$F_{IS} = -0.097^{NS}$	$F_{IS} = -0.163^{NS}$	$F_{IS} = 0.107^{NS}$	$F_{IS} = -0.016^{NS}$
Ballymoney	$H_O = 0.655$	$H_O = 0.367$	$H_O = 0.750$	$H_O = 0.690$	$H_O = 0.815$	$H_O = 0.633$	$H_O = 0.733$	$H_O = 0.933$
	$H_E = 0.630$	$H_E = 0.472$	$H_E = 0.886$	$H_E = 0.913$	$H_E = 0.853$	$H_E = 0.616$	$H_E = 0.873$	$H_E = 0.926$
	$F_{IS} = -0.040^{NS}$	$F_{IS} = 0.226^*$	$F_{IS} = 0.156^*$	$F_{IS} = 0.248^{***}$	$F_{IS} = 0.045^{NS}$	$F_{IS} = -0.028^{NS}$	$F_{IS} = 0.163^*$	$F_{IS} = -0.008^{NS}$
Peatlands Park	$H_O = 0.690$	$H_O = 0.345$	$H_O = 0.655$	$H_O = 0.536$	$H_O = 0.828$	$H_O = 0.464$	$H_O = 0.862$	$H_O = 0.964$
	$H_E = 0.626$	$H_E = 0.388$	$H_E = 0.814$	$H_E = 0.886$	$H_E = 0.840$	$H_E = 0.430$	$H_E = 0.909$	$H_E = 0.946$
	$F_{IS} = -0.105^{NS}$	$F_{IS} = 0.113^{NS}$	$F_{IS} = 0.198^*$	$F_{IS} = 0.400^{***}$	$F_{IS} = 0.015^{NS}$	$F_{IS} = -0.082^{NS}$	$F_{IS} = 0.052^{NS}$	$F_{IS} = -0.020^{NS}$
Carndaisy Woods	$H_O = 0.552$	$H_O = 0.367$	$H_O = 0.800$	$H_O = 0.767$	$H_O = 0.767$	$H_O = 0.667$	$H_O = 0.800$	$H_O = 0.800$
	$H_E = 0.657$	$H_E = 0.457$	$H_E = 0.891$	$H_E = 0.920$	$H_E = 0.864$	$H_E = 0.550$	$H_E = 0.924$	$H_E = 0.925$
	$F_{IS} = 0.163^{NS}$	$F_{IS} = 0.199^{NS}$	$F_{IS} = 0.104^{NS}$	$F_{IS} = 0.169^{**}$	$F_{IS} = 0.115^{NS}$	$F_{IS} = -0.216^{NS}$	$F_{IS} = 0.136^*$	$F_{IS} = 0.137^*$
Downhill	$H_O = 0.621$	$H_O = 0.414$	$H_O = 0.655$	$H_O = 0.556$	$H_O = 0.897$	$H_O = 0.517$	$H_O = 0.621$	$H_O = 0.966$
	$H_E = 0.719$	$H_E = 0.402$	$H_E = 0.910$	$H_E = 0.929$	$H_E = 0.905$	$H_E = 0.431$	$H_E = 0.873$	$H_E = 0.951$
	$F_{IS} = 0.139^{NS}$	$F_{IS} = -0.029^{NS}$	$F_{IS} = 0.284^{***}$	$F_{IS} = 0.406^{***}$	$F_{IS} = 0.010^{NS}$	$F_{IS} = -0.203^{NS}$	$F_{IS} = 0.293^{***}$	$F_{IS} = -0.016^{NS}$
Drum Manor	$H_O = 0.536$	$H_O = 0.467$	$H_O = 0.964$	$H_O = 0.867$	$H_O = 0.833$	$H_O = 0.586$	$H_O = 0.633$	$H_O = 0.929$
	$H_E = 0.622$	$H_E = 0.502$	$H_E = 0.913$	$H_E = 0.906$	$H_E = 0.851$	$H_E = 0.547$	$H_E = 0.933$	$H_E = 0.956$
	$F_{IS} = 0.141^{NS}$	$F_{IS} = 0.071^{NS}$	$F_{IS} = -0.057^{NS}$	$F_{IS} = 0.044^{NS}$	$F_{IS} = 0.021^{NS}$	$F_{IS} = -0.072^{NS}$	$F_{IS} = 0.325^{***}$	$F_{IS} = 0.029^{NS}$
Lemnagore Wood	$H_O = 0.586$	$H_O = 0.414$	$H_O = 0.964$	$H_O = 0.724$	$H_O = 0.929$	$H_O = 0.250$	$H_O = 0.846$	$H_O = 1.000$
	$H_E = 0.576$	$H_E = 0.413$	$H_E = 0.818$	$H_E = 0.906$	$H_E = 0.927$	$H_E = 0.265$	$H_E = 0.916$	$H_E = 0.939$
	$F_{IS} = -0.018^{NS}$	$F_{IS} = -0.001^{NS}$	$F_{IS} = -0.183^{NS}$	$F_{IS} = 0.204^{**}$	$F_{IS} = -0.002^{NS}$	$F_{IS} = 0.057^{NS}$	$F_{IS} = 0.077^{NS}$	$F_{IS} = -0.066^{NS}$

Table S3 (cont.)

Population	Locus							
	FR639485	FR646655	Femsatl-4	Femsatl-8	Femsatl-11	Femsatl-16	Femsatl-19	M230
Roe Valley	$H_O = 0.533$	$H_O = 0.533$	$H_O = 0.778$	$H_O = 0.679$	$H_O = 0.893$	$H_O = 0.400$	$H_O = 0.571$	$H_O = 0.966$
	$H_E = 0.642$	$H_E = 0.596$	$H_E = 0.882$	$H_E = 0.918$	$H_E = 0.907$	$H_E = 0.451$	$H_E = 0.873$	$H_E = 0.949$
	$F_{IS} = 0.172^{NS}$	$F_{IS} = 0.107^{NS}$	$F_{IS} = 0.120^{NS}$	$F_{IS} = 0.265^{***}$	$F_{IS} = 0.016^{NS}$	$F_{IS} = 0.116^{NS}$	$F_{IS} = 0.350^{***}$	$F_{IS} = -0.018^{NS}$
Knockmany Forest	$H_O = 0.833$	$H_O = 0.567$	$H_O = 0.933$	$H_O = 0.700$	$H_O = 0.833$	$H_O = 0.667$	$H_O = 0.900$	$H_O = 0.967$
	$H_E = 0.669$	$H_E = 0.455$	$H_E = 0.916$	$H_E = 0.918$	$H_E = 0.911$	$H_E = 0.564$	$H_E = 0.889$	$H_E = 0.933$
	$F_{IS} = -0.250^{NS}$	$F_{IS} = -0.250^{NS}$	$F_{IS} = -0.019^{NS}$	$F_{IS} = 0.240^{***}$	$F_{IS} = 0.087^{NS}$	$F_{IS} = -0.186^{NS}$	$F_{IS} = -0.013^{NS}$	$F_{IS} = -0.036^{NS}$
Slieve Beagh	$H_O = 0.643$	$H_O = 0.536$	$H_O = 0.786$	$H_O = 0.750$	$H_O = 0.750$	$H_O = 0.357$	$H_O = 0.679$	$H_O = 0.857$
	$H_E = 0.599$	$H_E = 0.497$	$H_E = 0.873$	$H_E = 0.931$	$H_E = 0.818$	$H_E = 0.317$	$H_E = 0.898$	$H_E = 0.932$
	$F_{IS} = -0.075^{NS}$	$F_{IS} = -0.079^{NS}$	$F_{IS} = 0.102^{NS}$	$F_{IS} = 0.197^{**}$	$F_{IS} = 0.085^{NS}$	$F_{IS} = -0.130^{NS}$	$F_{IS} = 0.248^{***}$	$F_{IS} = 0.082^{NS}$
Stranbane Glen	$H_O = 0.483$	$H_O = 0.433$	$H_O = 0.900$	$H_O = 0.793$	$H_O = 0.862$	$H_O = 0.467$	$H_O = 0.833$	$H_O = 0.897$
	$H_E = 0.606$	$H_E = 0.553$	$H_E = 0.888$	$H_E = 0.922$	$H_E = 0.909$	$H_E = 0.448$	$H_E = 0.904$	$H_E = 0.951$
	$F_{IS} = 0.206^{NS}$	$F_{IS} = 0.219^{NS}$	$F_{IS} = -0.014^{NS}$	$F_{IS} = 0.142^{*}$	$F_{IS} = 0.053^{NS}$	$F_{IS} = -0.042^{NS}$	$F_{IS} = 0.079^{NS}$	$F_{IS} = 0.058^{NS}$
Crom	$H_O = 0.552$	$H_O = 0.500$	$H_O = 0.833$	$H_O = 0.679$	$H_O = 0.724$	$H_O = 0.400$	$H_O = 0.867$	$H_O = 0.800$
	$H_E = 0.621$	$H_E = 0.460$	$H_E = 0.891$	$H_E = 0.939$	$H_E = 0.836$	$H_E = 0.456$	$H_E = 0.897$	$H_E = 0.941$
	$F_{IS} = 0.113^{NS}$	$F_{IS} = -0.088^{NS}$	$F_{IS} = 0.066^{NS}$	$F_{IS} = 0.281^{***}$	$F_{IS} = 0.137^{NS}$	$F_{IS} = 0.125^{NS}$	$F_{IS} = 0.034^{NS}$	$F_{IS} = 0.152^{**}$
Knockninny Hill	$H_O = 0.607$	$H_O = 0.393$	$H_O = 1.000$	$H_O = 0.464$	$H_O = 0.929$	$H_O = 0.429$	$H_O = 0.821$	$H_O = 0.889$
	$H_E = 0.642$	$H_E = 0.384$	$H_E = 0.929$	$H_E = 0.919$	$H_E = 0.893$	$H_E = 0.525$	$H_E = 0.907$	$H_E = 0.957$
	$F_{IS} = 0.055^{NS}$	$F_{IS} = -0.024^{NS}$	$F_{IS} = -0.078^{NS}$	$F_{IS} = 0.500^{***}$	$F_{IS} = -0.041^{NS}$	$F_{IS} = 0.187^{NS}$	$F_{IS} = 0.096^{NS}$	$F_{IS} = 0.072^{NS}$
Sloughan Glen	$H_O = 0.690$	$H_O = 0.483$	$H_O = 0.897$	$H_O = 0.655$	$H_O = 0.862$	$H_O = 0.414$	$H_O = 0.714$	$H_O = 0.862$
	$H_E = 0.584$	$H_E = 0.451$	$H_E = 0.874$	$H_E = 0.909$	$H_E = 0.855$	$H_E = 0.453$	$H_E = 0.898$	$H_E = 0.935$
	$F_{IS} = -0.184^{NS}$	$F_{IS} = -0.073^{NS}$	$F_{IS} = -0.027^{NS}$	$F_{IS} = 0.283^{***}$	$F_{IS} = -0.008^{NS}$	$F_{IS} = 0.088^{NS}$	$F_{IS} = 0.208^{**}$	$F_{IS} = 0.080^{NS}$

Table S3 (cont.)

Population	Locus							
	FR639485	FR646655	Femsatl-4	Femsatl-8	Femsatl-11	Femsatl-16	Femsatl-19	M230
Castle Hill	$H_O = 0.467$	$H_O = 0.200$	$H_O = 0.800$	$H_O = 0.633$	$H_O = 0.833$	$H_O = 0.467$	$H_O = 0.867$	$H_O = 0.828$
	$H_E = 0.704$	$H_E = 0.367$	$H_E = 0.866$	$H_E = 0.903$	$H_E = 0.877$	$H_E = 0.471$	$H_E = 0.911$	$H_E = 0.955$
	$F_{IS} = 0.341^{**}$	$F_{IS} = 0.461^{**}$	$F_{IS} = 0.078^{NS}$	$F_{IS} = 0.302^{***}$	$F_{IS} = 0.051^{NS}$	$F_{IS} = 0.009^{NS}$	$F_{IS} = 0.049^{NS}$	$F_{IS} = 0.135^{**}$
Ely Lodge	$H_O = 0.621$	$H_O = 0.483$	$H_O = 0.900$	$H_O = 0.724$	$H_O = 0.800$	$H_O = 0.433$	$H_O = 0.900$	$H_O = 0.964$
	$H_E = 0.650$	$H_E = 0.550$	$H_E = 0.912$	$H_E = 0.939$	$H_E = 0.911$	$H_E = 0.419$	$H_E = 0.904$	$H_E = 0.920$
	$F_{IS} = 0.045^{NS}$	$F_{IS} = 0.124^{NS}$	$F_{IS} = 0.013^{NS}$	$F_{IS} = 0.232^{***}$	$F_{IS} = 0.124^{NS}$	$F_{IS} = -0.036^{NS}$	$F_{IS} = 0.004^{NS}$	$F_{IS} = -0.049^{NS}$
Killeter Forest	$H_O = 0.793$	$H_O = 0.467$	$H_O = 0.767$	$H_O = 0.857$	$H_O = 0.862$	$H_O = 0.567$	$H_O = 0.724$	$H_O = 0.867$
	$H_E = 0.663$	$H_E = 0.445$	$H_E = 0.849$	$H_E = 0.924$	$H_E = 0.903$	$H_E = 0.547$	$H_E = 0.935$	$H_E = 0.964$
	$F_{IS} = -0.200^{NS}$	$F_{IS} = -0.050^{NS}$	$F_{IS} = 0.098^{NS}$	$F_{IS} = 0.074^{NS}$	$F_{IS} = 0.046^{NS}$	$F_{IS} = -0.036^{NS}$	$F_{IS} = 0.228^{***}$	$F_{IS} = 0.102^*$
Marble Arch	$H_O = 0.522$	$H_O = 0.259$	$H_O = 0.893$	$H_O = 0.808$	$H_O = 0.857$	$H_O = 0.571$	$H_O = 0.889$	$H_O = 0.885$
	$H_E = 0.610$	$H_E = 0.349$	$H_E = 0.888$	$H_E = 0.937$	$H_E = 0.866$	$H_E = 0.598$	$H_E = 0.918$	$H_E = 0.941$
	$F_{IS} = 0.147^{NS}$	$F_{IS} = 0.262^{NS}$	$F_{IS} = -0.005^{NS}$	$F_{IS} = 0.140^*$	$F_{IS} = 0.010^{NS}$	$F_{IS} = 0.045^{NS}$	$F_{IS} = 0.032^{NS}$	$F_{IS} = 0.061^{NS}$
Castle Caldwell	$H_O = 0.724$	$H_O = 0.172$	$H_O = 0.897$	$H_O = 0.533$	$H_O = 0.867$	$H_O = 0.533$	$H_O = 0.900$	$H_O = 0.867$
	$H_E = 0.665$	$H_E = 0.226$	$H_E = 0.836$	$H_E = 0.936$	$H_E = 0.895$	$H_E = 0.517$	$H_E = 0.920$	$H_E = 0.937$
	$F_{IS} = -0.091^{NS}$	$F_{IS} = 0.241^{NS}$	$F_{IS} = -0.074^{NS}$	$F_{IS} = 0.434^{***}$	$F_{IS} = 0.033^{NS}$	$F_{IS} = -0.032^{NS}$	$F_{IS} = 0.022^{NS}$	$F_{IS} = 0.077^{NS}$
Glenasmole Valley	$H_O = 0.821$	$H_O = 0.500$	$H_O = 0.926$	$H_O = 0.750$	$H_O = 0.821$	$H_O = 0.500$	$H_O = 0.893$	$H_O = 0.885$
	$H_E = 0.738$	$H_E = 0.466$	$H_E = 0.897$	$H_E = 0.894$	$H_E = 0.894$	$H_E = 0.477$	$H_E = 0.898$	$H_E = 0.945$
	$F_{IS} = -0.116^{NS}$	$F_{IS} = -0.075^{NS}$	$F_{IS} = -0.033^{NS}$	$F_{IS} = 0.163^*$	$F_{IS} = 0.082^{NS}$	$F_{IS} = -0.050^{NS}$	$F_{IS} = 0.006^{NS}$	$F_{IS} = 0.065^{NS}$
Knocknamallavoge	$H_O = 0.828$	$H_O = 0.552$	$H_O = 0.724$	$H_O = 0.724$	$H_O = 0.759$	$H_O = 0.621$	$H_O = 0.828$	$H_O = 0.931$
	$H_E = 0.638$	$H_E = 0.757$	$H_E = 0.822$	$H_E = 0.910$	$H_E = 0.866$	$H_E = 0.614$	$H_E = 0.918$	$H_E = 0.891$
	$F_{IS} = -0.304^{NS}$	$F_{IS} = 0.274^*$	$F_{IS} = 0.121^{NS}$	$F_{IS} = 0.208^{**}$	$F_{IS} = 0.126^{NS}$	$F_{IS} = -0.011^{NS}$	$F_{IS} = 0.100^{NS}$	$F_{IS} = -0.046^{NS}$

Table S3 (cont.)

Population	Locus							
	FR639485	FR646655	Femsatl-4	Femsatl-8	Femsatl-11	Femsatl-16	Femsatl-19	M230
Letterfrack	$H_O = 0.423$	$H_O = 0.385$	$H_O = 0.800$	$H_O = 0.640$	$H_O = 0.885$	$H_O = 0.391$	$H_O = 0.818$	$H_O = 0.808$
	$H_E = 0.659$	$H_E = 0.465$	$H_E = 0.914$	$H_E = 0.825$	$H_E = 0.902$	$H_E = 0.580$	$H_E = 0.909$	$H_E = 0.911$
	$F_{IS} = 0.363^{**}$	$F_{IS} = 0.176^{NS}$	$F_{IS} = 0.127^*$	$F_{IS} = 0.228^{**}$	$F_{IS} = 0.020^{NS}$	$F_{IS} = 0.330^*$	$F_{IS} = 0.102^{NS}$	$F_{IS} = 0.115^{NS}$

Figure Legends

Fig. 1 Locations of sites sampled in this study. Numbers correspond to those in Table 1.

Fig. 2. Two woodland sites sampled at (a) Belfast, Northern Ireland (insert) showing tree cover from orthophotographs of (b) Barnett Demesne and c) Cregagh Glen. Adult trees that were sampled are shown as red dots and saplings as blue dots; surrounding trees, hedgerows and woodlands are clearly visible. North is aligned with the top of the page. Image © 07/08/2006 DigitalGlobe, Google Earth.

Fig. 3. Mantel test for isolation-by-distance (IBD) between populations including (above) and excluding (below) the three Republic of Ireland populations.

Fig. 4. Summary of identified seed dispersal events in Barnett Demesne. (a) Histogram showing dispersal distances in 10 m classes. Black shading indicates assignment of a maternal plant from a parent-pair. Grey shading shows assignment of a maternal plant to a single identified parent. (b) Distance and direction of identified dispersal events. Broken arrows represent dispersal distances of greater than 50 m (values given in parentheses).

Fig. S1 Network showing relationships between the eight cpSSR haplotypes observed. Each mutation is shown by a dash, with the locus and allele size change indicated. An alternative homoplasious linkage between haplotypes H2 and H7 is indicated by the dashed line.

Fig. S2 Correlograms of autocorrelation coefficient (θ ; y-axis) plotted against distance (x-axis). 95% confidence intervals are indicated by dashed red lines. Note that in some

correlograms, the first two distance intervals (0 – 50 m and 50 – 100 m) may be at a different scale to subsequent intervals.

Fig. S3 Example of large allele dropout in consecutive individuals at locus M230. Note that in both cases, the large allele has not been called by the genotyping software.





























